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(54) VOIE DE SYNTHÈSE BIOLOGIQUE DES GENES DES 1-
DESOXY-D-XYLULOSE

(54) GENES OF THE 1-DESOXY-D-XYLULOSE BIOSYNTHETIC
PATHWAY

(57) The invention relates to the 1-desoxy- D-xylulose- 5-phosphate reductoisomerase gene, the 1-desoxy- D-xylulose- 5-phosphate- synthase gene and the gcpE gene of the 1-desoxy- D-xylulose biosynthetic pathway and to their use for transforming vectors, host organisms and plants and for determining substances that inhibit this biosynthetic pathway.



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(21) Internationales Aktenzeichen: PCT/EP99/07055 (22) Internationales Anmeldedatum: 22. September 1999 (22.09.99) (30) Prioritätsdaten: 198 43 279.8 22. September 1998 (22.09.98) DE 199 23 567.8 21. Mai 1999 (21.05.99) DE (71)(72) Anmelder und Erfinder: JOMAA, Hassan [DE/DE]; Breslauer Strasse 24, D-35398 Gießen (DE). (74) Anwälte: PANTEN, Kirsten usw.; Reichel und Reichel, Park- strasse 13, D-60322 Frankfurt am Main (DE).		(81) Bestimmungsstaaten: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DK, DM , EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO Patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), eurasisches Patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), europäisches Patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI Patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Veröffentlicht <i>Ohne internationalen Recherchenbericht und erneut zu veröffentlichen nach Erhalt des Berichts.</i>	
(54) Title: GENES OF THE 1-DESOXY-D-XYLULOSE BIOSYNTHETIC PATHWAY (54) Bezeichnung: GENE DES 1-DESOXY-D-XYLULOSE-BIOSYNTHESEWEGS (57) Abstract The invention relates to the 1-desoxy- D-xylulose- 5-phosphate reductoisomerase gene, the 1-desoxy- D-xylulose- 5-phosphate-synthase gene and the gcpE gene of the 1-desoxy- D-xylulose biosynthetic pathway and to their use for transforming vectors, host organisms and plants and for determining substances that inhibit this biosynthetic pathway. (57) Zusammenfassung Die vorliegende Erfindung betrifft das 1-Desoxy- D-xylulose- 5-phosphatreduktisomerase -Gen, das 1-Desoxy- D-xylulose- 5-phosphat- Synthase- Gen und das gcpE-Gen des 1-Desoxy- D-xylulose- Biosynthesewegs und ihre Verwendung zur Transformation von Vektoren, Wirtsorganismen und Pflanzen und zur Bestimmung von Stoffen, die diesen Biosyntheseweg inhibieren.			

- 4 -

Claims

1. DNA sequences which code for a polypeptide with the amino acid sequence shown in SEQ ID no. 2 or for an analogue or derivative of the polypeptide according to SEQ ID no. 2, in which one or more amino acids have been deleted, added or replaced by other amino acids, wherein the enzymatic action of the polypeptide is retained, and which sequences originate from parasites, wherein sequence variations occurring within the framework of natural strain variability are included.
2. DNA sequences which code for a polypeptide with the amino acid sequence shown in SEQ ID no. 4 or for an analogue or derivative of the polypeptide according to SEQ ID no. 4, in which one or more amino acids have been deleted, added or replaced by other amino acids, wherein the enzymatic action of the polypeptide is retained, and which sequences originate from parasites, wherein sequence variations occurring within the framework of natural strain variability are included.
3. DNA sequences which code for a polypeptide with the amino acid sequence shown in SEQ ID no. 6 or for an analogue or derivative of the polypeptide according to SEQ ID no. 6, in which one or more amino acids have been deleted, added or replaced by other amino acids wherein the catalytic function of the polypeptide is retained.

- 8 -

4. DNA sequence according to one of claims 1 to 3, characterised in that it also comprises functional regulation signals, in particular promoters, operators, enhancers, ribosomal binding sites.

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5. DNA sequence with the following sub-sequences

i) promoter which is active in viruses, eukaryotes and prokaryotes and ensures the formation of an RNA in the intended target tissue or target cells,

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ii) DNA sequences according to one of claims 1 to 3,

iii) 3' untranslated sequence which, in viruses, eukaryotes and prokaryotes, results in the addition of poly(A) residues onto the 3' end of the RNA.

15

6. Process for the production of transgenic viruses, eukaryotes and prokaryotes for modifying the isoprenoid content, characterised in that a DNA sequence according to claim 4 or 5 is transferred and incorporated into the genome of viruses, eukaryotic and prokaryotic cells with or without use of a vector.

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7. Transgenic systems, in particular plants and plant cells which contain one or more DNA sequences according to claims 1 to 5 as "foreign" or "additional" DNA, which sequences are expressed.

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8. Expression vector containing one or more DNA sequences according to claims 1 to 5.

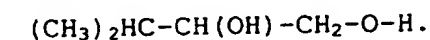
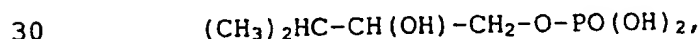
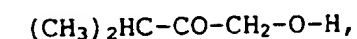
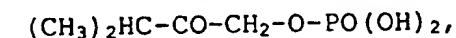
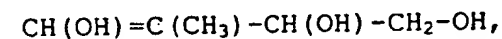
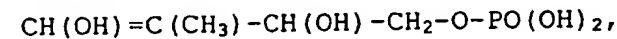
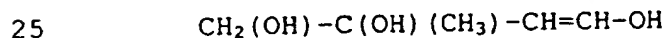
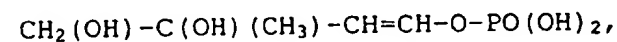
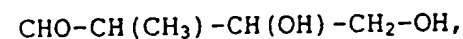
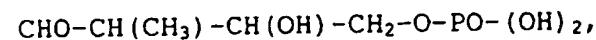
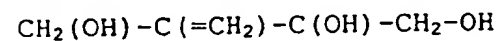
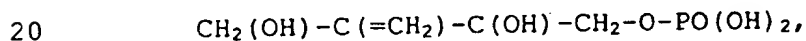
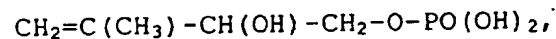
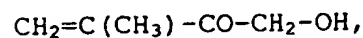
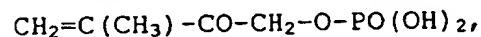
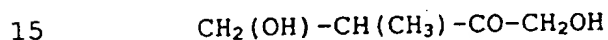
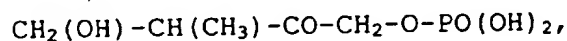
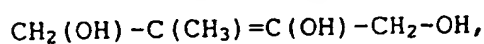
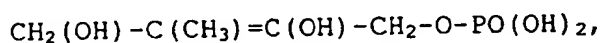
-ii-

9. Protein which is involved in the 1-deoxy-D-xylulose 5-phosphate metabolic pathway and a) is coded by DNA sequences SEQ ID no. 1, 3 or 5 or b) is coded by DNA sequences which hybridise with DNA sequences SEQ ID no. 1, 3, 5 or fragments of these DNA sequences in the DNA region which codes for the mature protein.
10. Protein according to claim 9, obtainable from the culture supernatants of parasites or from the disrupted parasites and purification by chromatographic and electrophoretic methods.
11. Protein according to one of claims 9 and 10, characterised in that it a) is the product of viral, prokaryotic or eukaryotic expression of exogenous DNA, b) is coded by sequences SEQ ID no. 1, 3 or 5 or is coded by DNA sequences which hybridise with DNA sequences SEQ ID no. 1, 3, 5 or fragments of these DNA sequences in the DNA region which codes for the mature protein, or c) is coded by DNA sequences which would hybridise without degeneration of the genetic code with the sequences defined in b) and which code for a polypeptide with a corresponding amino acid sequence.
12. Protein according to one of the preceding claims, characterised in that it comprises the amino acid sequences SEQ ID no. 2, 4 or 6.
13. Process for determining the enzymatic activity of the gcpe protein, characterised in that phosphorylation of a sugar or of a phosphorus sugar or of a precursor of isoprenoid biosynthesis, in

- 9 -

particular the phosphorylation of 2-C-methyl-D-erythritol, 2-C-methyl-D-erythritol phosphate, in particular 2-C-methyl-D-erythritol 4-phosphate, 2-C-methyl-D-erythrose, 2-C-methyl-D-erythrose phosphate, in particular 2-C-methyl-D-erythrose 4-phosphate, and of phosphate and alcohol precursors, is detected.

14. Process according to claim 13, characterised in that phosphorylation of the following phosphates or alcohols is detected:



- 7 -

15. Process for the combined determination of the enzymatic activity of DOXP synthase and of DOXP reductase, characterised in that the conversion of glyceraldehyde 3-phosphate into 2-C-methylerythritol 4-phosphate is detected.
16. Process for screening a compound for the treatment of infectious processes in humans and animals, wherein the process comprises:
- a) provision of a host cell which contains a recombinant expression vector, wherein the vector comprises at least a portion of the oligonucleotide sequence according to SEQ ID no. 1, SEQ ID no. 3 or SEQ ID no. 5 or variants or analogues thereof, and moreover of a compound suspected to have antimycotic, antibiotic, antiparasitic or antiviral action in humans and animals,
 - b) bringing the host cell into contact with the compound and
 - c) determining the antimicrobial, antimycotic, antibiotic, antiparasitic or antiviral action of the compound.
17. Process for screening for compounds for treating plants, wherein the process comprises:
- a) provision of a host cell which contains a recombinant expression vector, wherein the vector comprises at least a portion of the oligonucleotide sequence according to SEQ ID no. 1, SEQ ID no. 3 or SEQ ID no. 5 or variants or analogues thereof, and moreover of a compound suspected to have antimicrobial,

- 9 -

antiviral, antiparasitic, bactericidal,
fungicidal or herbicidal action in plants,

b) bringing the host cell into contact with the
compound and

5 c) determining the antimicrobial, antiviral,
antiparasitic, bactericidal, fungicidal or
herbicidal action of the compound.

10 18. Use of DNA according to one of claims 1 to 5 or of
proteins according to one of claims 9 to 12 or of
transgenic systems according to claim 7 for the
prevention or treatment of diseases in humans and
animals.

- 1 -

Genes of the 1-deoxy-D-xylulose biosynthesis pathway

The present invention relates to DNA sequences which, when incorporated into the genome of viruses, eukaryotes and prokaryotes, modify isoprenoid biosynthesis and to a genetic engineering process for the production of these transgenic viruses, eukaryotes and prokaryotes. The invention also relates to a process for the identification of substances having herbicidal, antimicrobial, antiparasitic, antiviral, fungicidal, bactericidal action in plants and antimicrobial, antiparasitic, antimycotic, antibacterial and antiviral action in humans and animals.

The biosynthesis pathway for the formation of isoprenoids via the classical acetate/mevalonate pathway and an alternative mevalonate-independent biosynthesis pathway, the deoxy-D-xylulose phosphate pathway is already known (Rohmer, M., Knani, M., Simonin, P., Sutter, B. and Sahn, H. (1993): *Biochem. J.* 295: 517-524).

It is, however, not known how and by which pathways it is possible to bring about a change in the isoprenoid concentration in viruses, eukaryotes and prokaryotes by means of the deoxy-D-xylulose phosphate pathway. Figure 1 shows this biosynthesis pathway.

DNA sequences are consequently provided which code for 1-deoxy-D-xylulase 5-phosphate synthase (DOXP synthase), 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DOXP reductoisomerase) or the gcpE protein. All three genes and enzymes are involved in isoprenoid biosynthesis.

- 2 -

(Translator's comment: The portion at the beginning of the next paragraph enclosed in square brackets corresponds to the beginning of the sentence which finishes on page 2, line 1 of the original).

[The gcpE protein has a kinase function and catalyses the phosphorylation of a sugar or a phosphorus sugar or a precursor of isoprenoid biosynthesis, in particular the phosphorylation of 2-C-methyl-D-erythritol, 2-C-methyl-D-erythritol phosphate, in particular 2-C-methyl-D-erythritol 4-phosphate, 2-C-methyl-D-erythrose, 2-C-methyl-D-erythrose] phosphate, in particular 2-C-methyl-D-erythrose 4-phosphate. In the precursor of isoprenoid synthesis, the gcpE protein in particular catalyses the phosphorylation of the following substances:

$\text{CH}_2(\text{OH})-\text{C}(\text{CH}_3)=\text{C}(\text{OH})-\text{CH}_2-\text{O}-\text{PO}(\text{OH})_2,$
 $\text{CH}_2(\text{OH})-\text{C}(\text{CH}_3)=\text{C}(\text{OH})-\text{CH}_2-\text{OH},$
 $\text{CH}_2(\text{OH})-\text{CH}(\text{CH}_3)-\text{CO}-\text{CH}_2-\text{O}-\text{PO}(\text{OH})_2,$
 $\text{CH}_2(\text{OH})-\text{CH}(\text{CH}_3)-\text{CO}-\text{CH}_2\text{OH}$
 $\text{CH}_2=\text{C}(\text{CH}_3)-\text{CO}-\text{CH}_2-\text{O}-\text{PO}(\text{OH})_2,$
 $\text{CH}_2=\text{C}(\text{CH}_3)-\text{CO}-\text{CH}_2-\text{OH},$
 $\text{CH}_2=\text{C}(\text{CH}_3)-\text{CH}(\text{OH})-\text{CH}_2-\text{O}-\text{PO}(\text{OH})_2,$
 $\text{CH}_2=\text{C}(\text{CH}_3)-\text{CH}(\text{OH})-\text{CH}_2-\text{OH},$
 $\text{CH}_2(\text{OH})-\text{C}(=\text{CH}_2)-\text{C}(\text{OH})-\text{CH}_2-\text{O}-\text{PO}(\text{OH})_2,$
 $\text{CH}_2(\text{OH})-\text{C}(=\text{CH}_2)-\text{C}(\text{OH})-\text{CH}_2-\text{OH}$
 $\text{CHO}-\text{CH}(\text{CH}_3)-\text{CH}(\text{OH})-\text{CH}_2-\text{O}-\text{PO}(\text{OH})_2,$
 $\text{CHO}-\text{CH}(\text{CH}_3)-\text{CH}(\text{OH})-\text{CH}_2-\text{OH},$
 $\text{CH}_2(\text{OH})-\text{C}(\text{OH})(\text{CH}_3)-\text{CH}=\text{CH}-\text{O}-\text{PO}(\text{OH})_2,$
 $\text{CH}_2(\text{OH})-\text{C}(\text{OH})(\text{CH}_3)-\text{CH}=\text{CH}-\text{OH}$
 $\text{CH}(\text{OH})=\text{C}(\text{CH}_3)-\text{CH}(\text{OH})-\text{CH}_2-\text{O}-\text{PO}(\text{OH})_2,$
 $\text{CH}(\text{OH})=\text{C}(\text{CH}_3)-\text{CH}(\text{OH})-\text{CH}_2-\text{OH},$
 $(\text{CH}_3)_2\text{HC}-\text{CO}-\text{CH}_2-\text{O}-\text{PO}(\text{OH})_2,$
 $(\text{CH}_3)_2\text{HC}-\text{CO}-\text{CH}_2-\text{O}-\text{H},$
 $(\text{CH}_3)_2\text{HC}-\text{CH}(\text{OH})-\text{CH}_2-\text{O}-\text{PO}(\text{OH})_2,$
 $(\text{CH}_3)_2\text{HC}-\text{CH}(\text{OH})-\text{CH}_2-\text{O}-\text{H}.$

- 2 -

DOXP synthase catalyses the condensation of pyruvate and
glyceraldehyde 3-phosphate to yield 1-deoxy-D-xylulose
5-phosphate and DOXP reductoisomerase catalyses the
5 conversion of 1-deoxy-D-xylulose 5-phosphate into
2-C-methyl-D-erythritol 4-phosphate (c.f. Fig. 1).

The invention relates to the following DNA sequences:
DNA sequences which code for a polypeptide with the amino
10 acid sequence shown in SEQ ID no. 2 or for an analogue or
derivative of the polypeptide according to SEQ ID no. 2,
in which one or more amino acids have been deleted, added
or replaced by other amino acids, wherein the enzymatic
action of the polypeptide is retained, and which
15 sequences originate from parasites, wherein sequence
variations occurring within the framework of natural
strain variability are included,

DNA sequences which code for a polypeptide with the amino
20 acid sequence shown in SEQ ID no. 4 or for an analogue or
derivative of the polypeptide according to SEQ ID no. 4,
in which one or more amino acids have been deleted, added
or replaced by other amino acids, wherein the enzymatic
action of the polypeptide is retained, and which
25 sequences originate from parasites, wherein sequence
variations occurring within the framework of natural
strain variability are included,

and DNA sequences which code for a polypeptide with the
30 amino acid sequence shown in SEQ ID no. 6 or for an
analogue or derivative of the polypeptide according to
SEQ ID no. 6, in which one or more amino acids have been

WO 00/17233

Amendments

PCT/EP99/07055

- 3 -

deleted, added or replaced by other amino acids, wherein the catalytic function of the polypeptide is retained.

25 . The genes and the gene products thereof (polypeptides)
are shown with their primary structure and are assigned
as follows:

SEQ ID no. 1: 1-deoxy-D-xylulose 5-phosphate reducto-
isomerase gene

30 SEQ ID no. 2: 1-deoxy-D-xylulose 5-phosphate reducto-
isomerase

SEQ ID no. 3: 1-deoxy-D-xylulose 5-phosphate synthase
gene

SEQ ID no. 4: 1-deoxy-D-xylulose 5-phosphate synthase

WO 00/17233

PCT/EP99/07055

- 4 -

SEQ ID no. 5: gcpE gene
SEQ ID no. 6: gcpE proteins.

5 The DNA sequences all originate from *Plasmodium falciparum*.

10 Apart from the DNA sequences stated in the sequence listing, suitable sequences are also those which, as a result of the degeneration of the genetic code, have another DNA sequence, but code for the same peptide or for an analogue or derivative of the polypeptide, in which one or more amino acids have been deleted, added or replaced by other amino acids.

15 The sequences according to the invention are suitable for the expression of genes in viruses, eukaryotes and prokaryotes which are responsible for isoprenoid biosynthesis in the 1-deoxy-D-xylulose pathway.

20 According to the invention, eukaryotes or eukaryotic cells include animal cells, plant cells, algae, yeasts, fungi, while prokaryotes or prokaryotic cells include bacteria, archaeobacteria and eubacteria.

25 When a DNA sequence is incorporated into a genome on which the above-stated DNA sequence is located, expression of the above-described genes in viruses, eukaryotes and prokaryotes is enabled. The viruses, eukaryotes and prokaryotes transformed according to the
30 invention are cultivated in a manner known per se and the isoprenoid formed during such cultivation is isolated and optionally purified. Not all isoprenoids need to be

- 5 -

isolated as in some case the isoprenoids are released directly into the ambient air.

The invention furthermore relates to a process for the production of transgenic viruses, eukaryotes and prokaryotes in order to modify the isoprenoid content, which process comprises the following steps.

- a) Production of a DNA sequence with the following sub-sequences
 - i) promoter which is active in viruses, eukaryotes and prokaryotes and ensures the formation of an RNA in the intended target tissue or target cells,
 - ii) DNA sequence which codes for a polypeptide with the amino acid sequence shown in SEQ ID no. 2, 4 or 6 or for an analogue or derivative of the polypeptide according to SEQ ID no. 2, 4 or 6,
 - iii) 5' and 3' untranslated sequence which enables or enhances expression of the stated genes in viruses, eukaryotes and prokaryotes,
- b) transfer and incorporation of the DNA sequence into the genome of viruses, prokaryotic or eukaryotic cells with or without the use of a vector (for example plasmid, viral DNA).

The intact, whole plants may be regenerated from plant cells transformed in this manner.

The protein-coding sequences with the nucleotide sequences SEQ ID no. 1, SEQ ID no. 3 and SEQ ID no. 5 may be provided with a promoter which ensures transcription in certain organs or cells, which promoter is coupled in

- 6 -

sense orientation (3' end of the promoter to the 5' end of the coding sequence) to the sequence which codes the protein to be formed. A termination signal which determines termination of mRNA synthesis is attached to the 3' end of the coding sequence. In order to direct the protein which is to be expressed to certain subcellular compartments, such as chloroplasts, amyloplasts, mitochondria, vacuoles, cytosol or intercellular spaces, a further sequence which codes for a so-called signal sequence or a transit peptide may be inserted between the promoter and the coding sequence. In some cases, it is necessary to insert sequences which code for a signal at the COOH terminus of the protein. The sequence must be in the same reading frame as the coding sequence of the protein. A large number of cloning vectors is available in order to prepare for the introduction of the DNA sequences according to the invention into higher plants, which vectors contain a replication signal for *E. coli* and a marker which permits selection of the transformed cells. Depending upon the method by which desired genes are introduced into the plant, further DNA sequences may be required. If, for example, the Ti or Ri plasmid is used to transform the plant cells, at least one right border, but frequently the right border and left border of the Ti and Ri plasmid T-DNA must be inserted as a flanking region into the genes to be introduced. The use of T-DNA for transforming plant cells has been intensively investigated and comprehensively described in EP 120516; Hoekama in "The Binary Plant Vector System", Offset-drukkerij Kanters B.V. Alblasserdam (1985), chapter V; Fraley et al., *Crit.Rev.Plant Sci.* 4, 1-46 and An et al. (1985) *EMBO J.* 4, 277-287. Once the introduced DNA has been incorporated into the genome, it is

generally stable and is also retained in the descendants of the originally transformed cells. It normally contains a selection marker, which imparts to the transformed plant cells resistance to a biocide or an antibiotic, such as kanamycin, G 418, bleomycin, hygromycin or phosphinotricin and others. The particular marker used is thus intended to allow selection of transformed cells from cells lacking the inserted DNA.

Many techniques are available for introducing DNA into a plant. These techniques include transformation with the assistance of agrobacteria, for example *Agrobacterium tumefaciens*, protoplast fusion, microinjection of DNA, electroporation, as well as ballistic methods and virus infection. Whole plants may then be regenerated from the transformed plant material in a suitable medium which may contain antibiotics or biocides for selection purposes. No particular requirements are placed upon the plasmids for injection and electroporation. However, if whole plants are to be regenerated from such transformed cells, a selectable marker gene must be present. The transformed cells grow in the plants in the conventional manner (McCormick et al. (1986), *Plant Cell Reports* 5, 81-84). The plants may be cultivated normally and be crossed with plants which have the same transformed genome or other genomes. The resultant individuals have the corresponding phenotypic properties.

The present invention also provides expression vectors which contain one or more of the DNA sequences according to the invention. Such expression vectors are obtained by providing the DNA sequences according to the invention with suitable functional regulation signals. Such

- 8 -

regulation signals are DNA sequences which are responsible for expression, for example promoters, operators, enhancers, ribosomal binding sites, and are recognised by the host organism.

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Further regulation signals, which for example control replication or recombination of the recombinant DNA in the host organism, may optionally also be a constituent part of the expression vector.

10

The host organisms transformed with the DNA sequences or expression vectors according to the invention are also provided by the present invention.

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Suitable host cells and organisms for expressing the enzymes according to the invention are those which comprise no intrinsic enzymes with the function of DOXP synthase, DOXP reductoisomerase or the gcpE protein. This is the case for archaeobacteria, animals, fungi, slime moulds and some eubacteria. The absence of such intrinsic enzyme activity substantially facilitates detection and purification of the recombinant enzymes. As a consequence, it is also for the first time possible straightforwardly to measure, in crude extracts from the host cells, the activity and in particular the inhibition of the activity of the recombinant enzymes according to the invention by various chemicals and pharmaceuticals.

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The enzymes according to the invention are advantageously then expressed in eukaryotic cells if post-translational modification and native folding of the polypeptide chain is to be achieved. Moreover, depending upon the expression system, it is ensured when expressing genomic

- 9 -

DNA sequences that introns are eliminated by splicing the DNA and the enzymes are produced in the polypeptide sequences characteristic to the parasites. Using recombinant DNA techniques, sequences coding for introns
5 may be eliminated from or inserted for experimental purposes into the DNA sequences to be expressed.

The protein may be isolated from the host cell or the culture supernatant of the host cell using methods known
10 to the person skilled in the art. In vitro reactivation of the enzymes may also be required.

In order to facilitate purification, the enzymes according to the invention or sub-sequences of the
15 enzymes may be expressed as fusion proteins with various peptide chains. Oligo-histidine sequences and sequences derived from glutathione S-transferase, thioredoxin or calmodulin-binding peptides are particularly suitable for this purpose.

20 The enzymes according to the invention or sub-sequences of the enzymes may furthermore be expressed as fusion proteins with such peptide chains known to the person skilled in the art that the recombinant enzymes are
25 transported into the extracellular medium or into certain compartments of the host cells. Both purification and investigation of the biological activity of the enzymes may consequently be facilitated.

30 When expressing the enzymes according to the invention, it may prove convenient to modify individual codons. Purposeful replacement of bases in the coding region may here also be advisable if the codons used in the

- 10 -

parasites differ from the codon use in the heterologous expression system, in order to ensure optimal synthesis of the protein.

5 The enzymes according to the invention may furthermore be obtained under standardised conditions by *in vitro* translation by methods known to the person skilled in the art. Systems suitable for this purpose are rabbit reticulocyte and wheat germ extracts and bacterial
10 lysates. *In vitro* transcribed mRNA may also be translated into *Xenopus* oocytes.

Oligo- and polypeptides, the sequences of which are derived from the peptide sequence of the enzymes
15 according to the invention, may be obtained by chemical synthesis. Given appropriate selection of the sequences, such peptides have properties which are characteristic of the enzymes according to the invention. Such peptides may be produced in large quantities and are particularly
20 suitable for investigating the kinetics of enzyme activity, regulation of enzyme activity, the three-dimensional structure of the enzymes, inhibition of enzyme activity by various chemicals and pharmaceuticals and the binding geometry and binding affinity of various
25 ligands.

DNA with the nucleotides from sequences SEQ ID no. 1, 3 and 5 are preferably used for the recombinant production of the enzymes according to the invention.
30

The invention accordingly moreover relates to a process for screening for compounds which inhibit the deoxy-D-xylulose phosphate metabolic pathway. According to this

- 11 -

process, a host organism, which contains a recombinant expression vector, wherein the vector comprises at least a portion of the oligonucleotide sequence according to SEQ ID no. 1, SEQ ID no. 3 or SEQ ID no. 5 or variants or homologues thereof, is provided, as is a compound which is suspected to have antimicrobial, antiparasitic, antibacterial, antiviral and antimycotic action in humans and animals or an antimicrobial, antiviral, bactericidal, herbicidal or fungicidal activity in plants. The host organism is then brought into contact with the compound and the activity of the compound determined.

The present invention also provides methods for determining the enzymatic activity of the gcpE protein. Said activity may be determined using known methods. Determination is performed by detecting the phosphorylation of a sugar or of a phosphorus sugar or of a precursor of isoprenoid biosynthesis, in particular the phosphorylation of 2-C-methyl-D-erythritol, 2-C-methyl-D-erythritol phosphate, in particular 2-C-methyl-D-erythritol 4-phosphate, 2-C-methyl-D-erythrose, 2-C-methyl-D-erythrose phosphate, in particular 2-C-methyl-D-erythrose 4-phosphate. The present invention also provides the use of this measurement method for identifying substances which inhibit the activity of the particular enzymes.

The enzymatic activity of DOXP synthase and DOXP reductoisomerase may be detected in a single step by determining the conversion of glyceraldehyde 3-phosphate into 2-C-methylerythritol 4-phosphate.

- 12 -

Determination of the activities of DOXP synthase and DOXP reductoisomerase proceeds analogously. Fluorimetric methods described by Querol et al. are also suitable for determining DOXP synthase activity (Querol et al.,
5 abstracts, 4th European Symposium on Plant Isoprenoids, Barcelona, 21-23 April 1999).

WO 00/17233

PCT/EP99/07055

- 1 -

SEQUENCE LISTING

<110> Jomaa, Hassan

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<140> PCT/EP99

<141> 1999-09-22

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WO 00/17233

- 3 -

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WO 00/17233

PCT/EP99/07055

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WO 00/17233

PCT/EP99/07055

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WO 00/17233

PCT/EP99/07055

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WO 00/17233

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WO 00/17233

PCT/EP99/07055

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tta gat aag gac cat tct gtg tat ata cgt ata ccc aga atg aac ata 3098
 Leu Asp Lys Asp His Ser Val Tyr Ile Arg Ile Pro Arg Met Asn Ile
 980 985 990

tta agt gat aag tac atg aaa gga tat ttg aac att cat atg aaa aat 3146
 Leu Ser Asp Lys Tyr Met Lys Gly Tyr Leu Asn Ile His Met Lys Asn
 995 1000 1005

gag agc aaa aat atc gat gta aac gtg gat ata aac gat gat gta gat 3194
 Glu Ser Lys Asn Ile Asp Val Asn Val Asp Ile Asn Asp Asp Val Asp
 1010 1015 1020

aaa tat agt gaa gaa tat atg gac gat gat aat ttt ata aaa tct ttt 3242
 Lys Tyr Ser Glu Glu Tyr Met Asp Asp Asp Asn Phe Ile Lys Ser Phe
 1025 1030 1035

att gga aaa tct aga att att aaa atg gat aat gaa aat aat aat aca 3290
 Ile Gly Lys Ser Arg Ile Ile Lys Met Asp Asn Glu Asn Asn Asn Thr
 1040 1045 1050 1055

aat gaa cat tat tca agc aga gga gat aca cag aca aaa aaa aaa aaa 3338
 Asn Glu His Tyr Ser Ser Arg Gly Asp Thr Gln Thr Lys Lys Lys Lys
 1060 1065 1070

gtt tgt atc ttt aac atg ggt agt atg ctt ttt aat gta att aat gct 3360
 Val Cys Ile Phe Asn Met Gly Ser Met Leu Phe Asn Val Ile Asn Ala
 1075 1080 1085

ata aaa gaa att gaa aaa gaa caa tat att tca cat aat tat tct ttt 3434
 Ile Lys Glu Ile Glu Lys Glu Gln Tyr Ile Ser His Asn Tyr Ser Phe
 1090 1095 1100

tca att gtt gat atg ata ttt tta aat cct tta gat aaa aat atg ata 3482
 Ser Ile Val Asp Met Ile Phe Leu Asn Pro Leu Asp Lys Asn Met Ile
 1105 1110 1115

WO 00/17233

PCT/EP99/07055

- 11 -

gat cat gta ata aaa caa aat aaa cat caa tat tta att act tat gaa 3530
 Asp His Val Ile Lys Gln Asn Lys His Gln Tyr Leu Ile Thr Tyr Glu
 1120 1125 1130 1135

gat aat act ata ggt ggt ttt tct aca cat ttc aat aat tat tta ata 3578
 Asp Asn Thr Ile Gly Gly Phe Ser Thr His Phe Asn Asn Tyr Leu Ile
 1140 1145 1150

gaa aat aat tat att aca aaa cat aac tta tat gtt cat aat att tat 3626
 Glu Asn Asn Tyr Ile Thr Lys His Asn Leu Tyr Val His Asn Ile Tyr
 1155 1160 1165

tta tct aat gag cca att gaa cat gca tct ttt aag gat caa caa gaa 3674
 Leu Ser Asn Glu Pro Ile Glu His Ala Ser Phe Lys Asp Gln Gln Glu
 1170 1175 1180

gtc gtc aaa atg gat aaa tgt agt ctt gtc aat aga att aaa aat tat 3722
 Val Val Lys Met Asp Lys Cys Ser Leu Val Asn Arg Ile Lys Asn Tyr
 1185 1190 1195

ctt aaa aat aat cct aca tgatgtaaga taaatatata tttctaaaat 3770
 Leu Lys Asn Asn Pro Thr
 1200 1205

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Asn Gln Ile Lys Thr Glu Lys Ile Tyr Ile Lys Lys Leu Asn Arg Leu
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Ser Arg Lys Asn Ser Leu Cys Ser Ser Lys Asn Lys Ile Ala Cys Leu
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Phe Asp Ile Gly Asn Asp Asp Asn Arg Asn Thr Thr Tyr Gly Tyr Asn
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Val Asn Val Lys Asn Asp Asp Ile Asn Ser Leu Leu Lys Asn Asn Tyr
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Ser Asn Lys Leu Tyr Met Asp Lys Arg Lys Asn Ile Asn Asn Val Ile
 100 105 110

Ser Thr Asn Lys Ile Ser Gly Ser Ile Ser Asn Ile Cys Ser Arg Asn
 115 120 125

WO 00/17233

PCT/EP99/07055

- 12 -

Gln Lys Glu Asn Glu Gln Lys Arg Asn Lys Gln Arg Cys Leu Thr Gln
 130 135 140

Cys His Thr Tyr Asn Met Ser His Glu Gln Asp Lys Leu Ala Asn Asp
 145 150 155 160

Asn Asn Arg Asn Asn Lys Lys Asn Phe Asn Leu Leu Phe Ile Asn Tyr
 165 170 175

Phe Asn Leu Lys Arg Met Lys Asn Ser Leu Leu Asn Lys Asp Asn Phe
 180 185 190

Phe Tyr Cys Lys Glu Lys Lys Leu Ser Phe Leu His Lys Ala Tyr Lys
 195 200 205

Lys Lys Asn Cys Thr Phe Gln Asn Tyr Ser Leu Lys Arg Lys Ser Asn
 210 215 220

Arg Asp Ser His Lys Leu Phe Ser Gly Glu Phe Asp Asp Tyr Thr Asn
 225 230 235 240

Asn Asn Ala Leu Tyr Glu Ser Glu Lys Lys Glu Tyr Ile Thr Leu Asn
 245 250 255

Asn Asn Asn Lys Asn Asn Asn Asn Lys Asn Asn Asp Asn Lys Asn Asn
 260 265 270

Asp Asn Asn Asp Tyr Asn Asn Asn Asn Ser Cys Asn Asn Leu Gly Glu
 275 280 285

Arg Ser Asn His Tyr Asp Asn Tyr Gly Gly Asp Asn Asn Asn Pro Cys
 290 295 300

Asn Asn Asn Asn Asp Lys Tyr Asp Ile Gly Lys Tyr Phe Lys Gln Ile
 305 310 315 320

Asn Thr Phe Ile Asn Ile Asp Glu Tyr Lys Thr Ile Tyr Gly Asp Glu
 325 330 335

Ile Tyr Lys Glu Ile Tyr Glu Leu Tyr Val Glu Arg Asn Ile Pro Glu
 340 345 350

Tyr Tyr Glu Arg Lys Tyr Phe Ser Glu Asp Ile Lys Lys Ser Val Leu
 355 360 365

Phe Asp Ile Asp Lys Tyr Asn Asp Val Glu Phe Glu Lys Ala Ile Lys
 370 375 380

Glu Glu Phe Ile Asn Asn Gly Val Tyr Ile Asn Asn Ile Asp Asn Thr
 385 390 395 400

Tyr Tyr Lys Lys Glu Asn Ile Leu Ile Met Lys Lys Ile Leu His Tyr
 405 410 415

Phe Pro Leu Leu Lys Leu Ile Asn Asn Pro Ser Asp Leu Lys Lys Leu
 420 425 430

WO 00/17233

PCT/EP99/07055

- 13 -

Lys Lys Gln Tyr Leu Pro Leu Leu Ala His Glu Leu Lys Ile Phe Leu
 435 440 445
 Phe Phe Ile Val Asn Ile Thr Gly Gly His Phe Ser Ser Val Leu Ser
 450 455 460
 Ser Leu Glu Ile Gln Leu Leu Leu Tyr Ile Phe Asn Gln Pro Tyr
 465 470 475 480
 Asp Asn Val Ile Tyr Asp Ile Gly His Gln Ala Tyr Val His Lys Ile
 485 490 495
 Leu Thr Gly Arg Lys Leu Leu Phe Leu Ser Leu Arg Asn Lys Lys Gly
 500 505 510
 Ile Ser Gly Phe Leu Asn Ile Phe Glu Ser Ile Tyr Asp Lys Phe Gly
 515 520 525
 Ala Gly His Ser Ser Thr Ser Leu Ser Ala Ile Gln Gly Tyr Tyr Glu
 530 535 540
 Ala Glu Trp Gln Val Lys Asn Lys Glu Lys Tyr Gly Asn Gly Asp Ile
 545 550 555 560
 Glu Ile Ser Asp Asn Ala Asn Val Thr Asn Asn Glu Arg Ile Phe Gln
 565 570 575
 Lys Gly Ile His Asn Asp Asn Asn Ile Asn Asn Asn Ile Asn Asn Asn
 580 585 590
 Asn Tyr Ile Asn Pro Ser Asp Val Val Gly Arg Glu Asn Thr Asn Val
 595 600 605
 Pro Asn Val Arg Asn Asp Asn His Asn Val Asp Lys Val His Ile Ala
 610 615 620
 Ile Ile Gly Asp Gly Gly Leu Thr Gly Gly Met Ala Leu Glu Ala Leu
 625 630 635 640
 Asn Tyr Ile Ser Phe Leu Asn Ser Lys Ile Leu Ile Ile Tyr Asn Asp
 645 650 655
 Asn Gly Gln Val Ser Leu Pro Thr Asn Ala Val Ser Ile Ser Gly Asn
 660 665 670
 Arg Pro Ile Gly Ser Ile Ser Asp His Leu His Tyr Phe Val Ser Asn
 675 680 685
 Ile Glu Ala Asn Ala Gly Asp Asn Lys Leu Ser Lys Asn Ala Lys Glu
 690 695 700
 Asn Asn Ile Phe Glu Asn Leu Asn Tyr Asp Tyr Ile Gly Val Val Asn
 705 710 715 720
 Gly Asn Asn Thr Glu Glu Leu Phe Lys Val Leu Asn Asn Ile Lys Glu
 725 730 735

WO 00/17233

- 14 -

Asn Lys Leu Lys Arg Ala Thr Val Leu His Val Arg Thr Lys Lys Ser
 740 745 750

Asn Asp Phe Ile Asn Ser Lys Ser Pro Ile Ser Ile Leu His Ser Ile
 755 760 765

Lys Lys Asn Glu Ile Phe Pro Phe Asp Thr Thr Ile Leu Asn Gly Asn
 770 775 780

Ile His Lys Glu Asn Lys Ile Glu Glu Glu Lys Asn Val Ser Ser Ser
 785 790 795 800

Thr Lys Tyr Asp Val Asn Asn Lys Asn Asn Lys Asn Asn Asp Asn Ser
 805 810 815

Glu Ile Ile Lys Tyr Glu Asp Met Phe Ser Lys Glu Thr Phe Thr Asp
 820 825 830

Ile Tyr Thr Asn Glu Met Leu Lys Tyr Leu Lys Lys Asp Arg Asn Ile
 835 840 845

Ile Phe Leu Ser Pro Ala Met Leu Gly Gly Ser Gly Leu Val Lys Ile
 850 855 860

Ser Glu Arg Tyr Pro Asn Asn Val Tyr Asp Val Gly Ile Ala Glu Gln
 865 870 875 880

His Ser Val Thr Phe Ala Ala Ala Met Ala Met Asn Lys Lys Leu Lys
 885 890 895

Ile Gln Leu Cys Ile Tyr Ser Thr Phe Leu Gln Arg Ala Tyr Asp Gln
 900 905 910

Ile Ile His Asp Leu Asn Leu Gln Asn Ile Pro Leu Lys Val Ile Ile
 915 920 925

Gly Arg Ser Gly Leu Val Gly Glu Asp Gly Ala Thr His Gln Gly Ile
 930 935 940

Tyr Asp Leu Ser Tyr Leu Gly Thr Leu Asn Asn Ala Tyr Ile Ile Ser
 945 950 955 960

Pro Ser Asn Gln Val Asp Leu Lys Arg Ala Leu Arg Phe Ala Tyr Leu
 965 970 975

Asp Lys Asp His Ser Val Tyr Ile Arg Ile Pro Arg Met Asn Ile Leu
 980 985 990

Ser Asp Lys Tyr Met Lys Gly Tyr Leu Asn Ile His Met Lys Asn Glu
 995 1000 1005

Ser Lys Asn Ile Asp Val Asn Val Asp Ile Asn Asp Asp Val Asp Lys
 1010 1015 1020

Tyr Ser Glu Glu Tyr Met Asp Asp Asp Asn Phe Ile Lys Ser Phe Ile
 1025 1030 1035 1040

WO 00/17233

PCT/EP99/07055

- 15 -

Gly Lys Ser Arg Ile Ile Lys Met Asp Asn Glu Asn Asn Asn Thr Asn
 1045 1050 1055

Glu His Tyr Ser Ser Arg Gly Asp Thr Gln Thr Lys Lys Lys Lys Val
 1060 1065 1070

Cys Ile Phe Asn Met Gly Ser Met Leu Phe Asn Val Ile Asn Ala Ile
 1075 1080 1085

Lys Glu Ile Glu Lys Glu Gln Tyr Ile Ser His Asn Tyr Ser Phe Ser
 1090 1095 1100

Ile Val Asp Met Ile Phe Leu Asn Pro Leu Asp Lys Asn Met Ile Asp
 1105 1110 1115 1120

His Val Ile Lys Gln Asn Lys His Gln Tyr Leu Ile Thr Tyr Glu Asp
 1125 1130 1135

Asn Thr Ile Gly Gly Phe Ser Thr His Phe Asn Asn Tyr Leu Ile Glu
 1140 1145 1150

Asn Asn Tyr Ile Thr Lys His Asn Leu Tyr Val His Asn Ile Tyr Leu
 1155 1160 1165

Ser Asn Glu Pro Ile Glu His Ala Ser Phe Lys Asp Gln Gln Glu Val
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Val Lys Met Asp Lys Cys Ser Leu Val Asn Arg Ile Lys Asn Tyr Leu
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Lys Asn Asn Pro Thr
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tgccgaata accacaaa atg agt tat ata aaa aga ctg att ctt ttt atg 231
 Met Ser Tyr Ile Lys Arg Leu Ile Leu Phe Met
 1 5 10

tta ctg ttt tat tct cat gta aaa att aaa aaa tta ttt att aaa att 279
 Leu Leu Phe Tyr Ser His Val Lys Ile Lys Lys Leu Phe Ile Lys Ile
 15 20 25

WO 00/17233

PCT/EP99/07055

- 16 -

tct aat gta aac ata ttt ttt gca gaa gca aag aaa aat gga aaa aag Ser Asn Val Asn Ile Phe Phe Ala Glu Ala Lys Lys Asn Gly Lys Lys 30 35 40	327
gaa ttc ttt ctt ttt tta cta aat ata aaa aaa aat agc caa cag aaa Glu Phe Phe Leu Phe Leu Leu Asn Ile Lys Lys Asn Ser Gln Gln Lys 45 50 55	375
aaa act tat cat att acc aaa agg aat acc ata aat aaa agt gat ttt Lys Thr Tyr His Ile Thr Lys Arg Asn Thr Ile Asn Lys Ser Asp Phe 60 65 70 75	423
tta tat tct tta cta aat gaa gaa ggg aat tct tca aaa aag gaa tat Leu Tyr Ser Leu Leu Asn Glu Glu Gly Asn Ser Ser Lys Lys Glu Tyr 80 85 90	471
aaa aat tta aaa gat gaa gaa aaa tat aat atc ata caa aat ata aaa Lys Asn Leu Lys Asp Glu Glu Lys Tyr Asn Ile Ile Gln Asn Ile Lys 95 100 105	519
aaa tat tgt gaa tgt act aaa aaa tat aaa agg ctc cca aca cga gaa Lys Tyr Cys Glu Cys Thr Lys Lys Tyr Lys Arg Leu Pro Thr Arg Glu 110 115 120	567
gta gtt att gga aat gtt aaa att gga gga aat aat aaa ata gct att Val Val Ile Gly Asn Val Lys Ile Gly Gly Asn Asn Lys Ile Ala Ile 125 130 135	615
caa act atg gct agc tgt gat aca aga aat gta gaa gaa tgt gta tat Gln Thr Met Ala Ser Cys Asp Thr Arg Asn Val Glu Glu Cys Val Tyr 140 145 150 155	663
caa att aga aaa tgt aaa gat ttg ggt gct gac att gta agg ttg act Gln Ile Arg Lys Cys Lys Asp Leu Gly Ala Asp Ile Val Arg Leu Thr 160 165 170	711
gtt caa gga gtt caa gaa gca caa gct agt tat cat att aaa gaa aaa Val Gln Gly Val Gln Glu Ala Gln Ala Ser Tyr His Ile Lys Glu Lys 175 180 185	759
tta tta tct gaa aat gta aat atc cca tta gta gca gat att cat ttt Leu Leu Ser Glu Asn Val Asn Ile Pro Leu Val Ala Asp Ile His Phe 190 195 200	807
aat cct aaa ata gct tta atg gca gct gat gtg ttt gaa aaa att cga Asn Pro Lys Ile Ala Leu Met Ala Ala Asp Val Phe Glu Lys Ile Arg 205 210 215	855
gtg aat cca gga aat tat gtt gat gga aga aaa aaa tgg ata gat aaa Val Asn Pro Gly Asn Tyr Val Asp Gly Arg Lys Lys Trp Ile Asp Lys 220 225 230 235	903
gtt tat aaa aot aaa gaa gaa ttt gat gaa ggg aaa tta ttt ata aaa Val Tyr Lys Thr Lys Glu Glu Phe Asp Glu Gly Lys Leu Phe Ile Lys 240 245 250	951

WO 00/17233

PCT/EP99/07055

- 17 -

gaa aaa ttt gta cca tta att gaa aaa tgt aaa aga tta aat aga gca Glu Lys Phe Val Pro Leu Ile Glu Lys Cys Lys Arg Leu Asn Arg Ala 255 260 265	999
ata aga att gga aca aat cat gga tcc ctt tca tct cga gta tta tca Ile Arg Ile Gly Thr Asn His Gly Ser Leu Ser Ser Arg Val Leu Ser 270 275 280	1047
tat tat gga gat aca cca tta ggt atg gta gaa tgc gct ttt gag ttt Tyr Tyr Gly Asp Thr Pro Leu Gly Met Val Glu Ser Ala Phe Glu Phe 285 290 295	1095
tct gat tta tgt att gaa aac aat ttt tac aat ctt gtt ttt tct atg Ser Asp Leu Cys Ile Glu Asn Asn Phe Tyr Asn Leu Val Phe Ser Met 300 305 310 315	1143
aaa gct tct aat gct tat gtt atg ata caa tct tat aga tta tta gta Lys Ala Ser Asn Ala Tyr Val Met Ile Gln Ser Tyr Arg Leu Leu Val 320 325 330	1191
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tta aca gaa gat cct tgg gaa gag tta act cct tgt aaa aaa tta gtt Leu Thr Glu Asp Pro Trp Glu Glu Leu Thr Pro Cys Lys Lys Leu Val 380 385 390 395	1383
gaa aat tta aag aaa aga ata ttt tat aat gaa aat ttt aaa gaa gat Glu Asn Leu Lys Lys Arg Ile Phe Tyr Asn Glu Asn Phe Lys Glu Asp 400 405 410	1431
aat gaa tta aaa aat aat gaa atg gat acc aaa aat cta tta aat ttt Asn Glu Leu Lys Asn Asn Glu Met Asp Thr Lys Asn Leu Leu Asn Phe 415 420 425	1479
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aaa aat aat aat gta tta cat gaa gag tgc act ata ggt aat gta gta Lys Asn Asn Asn Val Leu His Glu Glu Cys Thr Ile Gly Asn Val Val 445 450 455	1575
acc ata aaa gag tta gaa gat tct ctg caa att ttt aaa gat tta aat Thr Ile Lys Glu Leu Glu Asp Ser Leu Gln Ile Phe Lys Asp Leu Asn 460 465 470 475	1623

WO 00/17233

PCT/EP99/07055

- 18 -

tta gaa gta gat tca aat gga aat ttg aaa aag gga gcc aaa aca act	1671
Leu Glu Val Asp Ser Asn Gly Asn Leu Lys Lys Gly Ala Lys Thr Thr	
480 485 490	
gat atg gtt att ata aat gat ttt cat aat ata aca aat tta gga aaa	1719
Asp Met Val Ile Ile Asn Asp Phe His Asn Ile Thr Asn Leu Gly Lys	
495 500 505	
aaa act gtg gat aaa tta atg caa gtg gga att aat ata gta gtt caa	1767
Lys Thr Val Asp Lys Leu Met Gln Val Gly Ile Asn Ile Val Val Gln	
510 515 520	
tat gaa cca cat aat ata gaa ttt ata gaa aaa atg gaa cca aat aat	1815
Tyr Glu Pro His Asn Ile Glu Phe Ile Glu Lys Met Glu Pro Asn Asn	
525 530 535	
gat aat aat aat aat aat aat aat aat aat ata tta ttt tat gtg gat	1863
Asp Asn Asn Asn Asn Asn Asn Asn Asn Asn Ile Leu Phe Tyr Val Asp	
540 545 550 555	
ata aaa aat att atg aac agt tca gaa aaa aat att aaa tta agt aat	1911
Ile Lys Asn Ile Met Asn Ser Ser Glu Lys Asn Ile Lys Leu Ser Asn	
560 565 570	
tct aaa gga tat gga tta att tta aac gga aaa gaa gat ata caa acc	1959
Ser Lys Gly Tyr Gly Leu Ile Leu Asn Gly Lys Glu Asp Ile Gln Thr	
575 580 585	
ata aaa aaa ata aaa gaa tta aat cgt cgt cct tta ttc att cta tta	2007
Ile Lys Lys Ile Lys Glu Leu Asn Arg Arg Pro Leu Phe Ile Leu Leu	
590 595 600	
aaa tca gat aac ata tat gaa cat gta tta ata acc aga aga att aat	2055
Lys Ser Asp Asn Ile Tyr Glu His Val Leu Ile Thr Arg Arg Ile Asn	
605 610 615	
gaa ctt tta caa tcc tta aat ata aat ata cct tat ata cat tat gtt	2103
Glu Leu Leu Gln Ser Leu Asn Ile Asn Ile Pro Tyr Ile His Tyr Val	
620 625 630 635	
gat att aat tca aac aat tat gat gat ata tta gtt aat tca aca tta	2151
Asp Ile Asn Ser Asn Asn Tyr Asp Asp Ile Leu Val Asn Ser Thr Leu	
640 645 650	
tat gca gga agt tgt ttg atg gat tta atg ggg gat ggt ctt att gtt	2199
Tyr Ala Gly Ser Cys Leu Met Asp Leu Met Gly Asp Gly Leu Ile Val	
655 660 665	
aac gta act aat gat gtt ctt aca aat aaa aaa aag ata gaa aca aaa	2247
Asn Val Thr Asn Asp Val Leu Thr Asn Lys Lys Lys Ile Glu Thr Lys	
670 675 680	
tat gat gaa aaa gaa gaa gta gag gaa gag gga aac aat aaa gat att	2295
Tyr Asp Glu Lys Glu Glu Val Glu Glu Glu Gly Asn Asn Lys Asp Ile	
685 690 695	

WO 00/17233

PCT/EP99/07055

- 19 -

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 Ile Leu Gln Asp Thr Arg Ile Arg Leu Phe Lys Thr Asp Tyr Ile Ala
 720 725 730
 tgc cca tct tgt gga aga act tta ttt aat ata caa gaa act act aaa 2439
 Cys Pro Ser Cys Gly Arg Thr Leu Phe Asn Ile Gln Glu Thr Thr Lys
 735 740 745
 aaa att atg aaa tta aca ggg cac tta aaa ggc gtt aaa att gca gtc 2487
 Lys Ile Met Lys Leu Thr Gly His Leu Lys Gly Val Lys Ile Ala Val
 750 755 760
 atg gga tgt att gtt aat ggt ata gga gaa atg gca gat gca cat ttt 2535
 Met Gly Cys Ile Val Asn Gly Ile Gly Glu Met Ala Asp Ala His Phe
 765 770 775
 ggt tat gtt ggt agt gca cct aaa aaa att gat tta tat tat ggt aaa 2583
 Gly Tyr Val Gly Ser Ala Pro Lys Lys Ile Asp Leu Tyr Tyr Gly Lys
 780 785 790 795
 gag tta gta gaa aga aat ata cct gag gaa gaa gct tgt gat aaa ttg 2631
 Glu Leu Val Glu Arg Asn Ile Pro Glu Glu Glu Ala Cys Asp Lys Leu
 800 805 810
 ata gaa tta att aaa aaa cat aac aaa tgg aaa gat cca taaattgaat 2680
 Ile Glu Leu Ile Lys Lys His Asn Lys Trp Lys Asp Pro
 815 820
 atggacaagt atttatttat ttatttatct tatatataat atattataaa tttttcgatg 2740
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<213> Plasmodium falciparum

<400> 6

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WO 00/17233

PCT/EP99/07055

- 20 -

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 20 25 30
 Phe Phe Ala Glu Ala Lys Lys Asn Gly Lys Lys Glu Phe Phe Leu Phe
 35 40 45
 Leu Leu Asn Ile Lys Lys Asn Ser Gln Gln Lys Lys Thr Tyr His Ile
 50 55 60
 Thr Lys Arg Asn Thr Ile Asn Lys Ser Asp Phe Leu Tyr Ser Leu Leu
 65 70 75 80
 Asn Glu Glu Gly Asn Ser Ser Lys Lys Glu Tyr Lys Asn Leu Lys Asp
 85 90 95
 Glu Glu Lys Tyr Asn Ile Ile Gln Asn Ile Lys Lys Tyr Cys Glu Cys
 100 105 110
 Thr Lys Lys Tyr Lys Arg Leu Pro Thr Arg Glu Val Val Ile Gly Asn
 115 120 125
 Val Lys Ile Gly Gly Asn Asn Lys Ile Ala Ile Gln Thr Met Ala Ser
 130 135 140
 Cys Asp Thr Arg Asn Val Glu Glu Cys Val Tyr Gln Ile Arg Lys Cys
 145 150 155 160
 Lys Asp Leu Gly Ala Asp Ile Val Arg Leu Thr Val Gln Gly Val Gln
 165 170 175
 Glu Ala Gln Ala Ser Tyr His Ile Lys Glu Lys Leu Leu Ser Glu Asn
 180 185 190
 Val Asn Ile Pro Leu Val Ala Asp Ile His Phe Asn Pro Lys Ile Ala
 195 200 205
 Leu Met Ala Ala Asp Val Phe Glu Lys Ile Arg Val Asn Pro Gly Asn
 210 215 220
 Tyr Val Asp Gly Arg Lys Lys Trp Ile Asp Lys Val Tyr Lys Thr Lys
 225 230 235 240
 Glu Glu Phe Asp Glu Gly Lys Leu Phe Ile Lys Glu Lys Phe Val Pro
 245 250 255
 Leu Ile Glu Lys Cys Lys Arg Leu Asn Arg Ala Ile Arg Ile Gly Thr
 260 265 270
 Asn His Gly Ser Leu Ser Ser Arg Val Leu Ser Tyr Tyr Gly Asp Thr
 275 280 285
 Pro Leu Gly Met Val Glu Ser Ala Phe Glu Phe Ser Asp Leu Cys Ile
 290 295 300
 Glu Asn Asn Phe Tyr Asn Leu Val Phe Ser Met Lys Ala Ser Asn Ala
 305 310 315 320
 Tyr Val Met Ile Gln Ser Tyr Arg Leu Leu Val Ser Lys Gln Tyr Glu

WO 00/17233

- 21 -

325 330 335
 Arg Asn Met Met Phe Pro Ile His Leu Gly Val Thr Glu Ala Gly Phe
 340 345 350
 Gly Asp Asn Gly Arg Ile Lys Ser Tyr Leu Gly Ile Gly Ser Leu Leu
 355 360 365
 Tyr Asp Gly Ile Gly Asp Thr Ile Arg Ile Ser Leu Thr Glu Asp Pro
 370 375 380
 Trp Glu Glu Leu Thr Pro Cys Lys Lys Leu Val Glu Asn Leu Lys Lys
 385 390 395 400
 Arg Ile Phe Tyr Asn Glu Asn Phe Lys Glu Asp Asn Glu Leu Lys Asn
 405 410 415
 Asn Glu Met Asp Thr Lys Asn Leu Leu Asn Phe Glu Glu Asn Tyr Arg
 420 425 430
 Asn Phe Asn Asn Ile Lys Lys Arg Asn Val Glu Lys Asn Asn Asn Val
 435 440 445
 Leu His Glu Glu Cys Thr Ile Gly Asn Val Val Thr Ile Lys Glu Leu
 450 455 460
 Glu Asp Ser Leu Gln Ile Phe Lys Asp Leu Asn Leu Glu Val Asp Ser
 465 470 475 480
 Asn Gly Asn Leu Lys Lys Gly Ala Lys Thr Thr Asp Met Val Ile Ile
 485 490 495
 Asn Asp Phe His Asn Ile Thr Asn Leu Gly Lys Lys Thr Val Asp Lys
 500 505 510
 Leu Met Gln Val Gly Ile Asn Ile Val Val Gln Tyr Glu Pro His Asn
 515 520 525
 Ile Glu Phe Ile Glu Lys Met Glu Pro Asn Asn Asp Asn Asn Asn Asn
 530 535 540
 Asn Asn Asn Asn Asn Ile Leu Phe Tyr Val Asp Ile Lys Asn Ile Met
 545 550 555 560
 Asn Ser Ser Glu Lys Asn Ile Lys Leu Ser Asn Ser Lys Gly Tyr Gly
 565 570 575
 Leu Ile Leu Asn Gly Lys Glu Asp Ile Gln Thr Ile Lys Lys Ile Lys
 580 585 590
 Glu Leu Asn Arg Arg Pro Leu Phe Ile Leu Leu Lys Ser Asp Asn Ile
 595 600 605
 Tyr Glu His Val Leu Ile Thr Arg Arg Ile Asn Glu Leu Leu Gln Ser
 610 615 620
 Leu Asn Ile Asn Ile Pro Tyr Ile His Tyr Val Asp Ile Asn Ser Asn
 625 630 635 640

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PCT/EP99/07055

- 22 -

Asn Tyr Asp Asp Ile Leu Val Asn Ser Thr Leu Tyr Ala Gly Ser Cys
 645 650 655

Leu Met Asp Leu Met Gly Asp Gly Leu Ile Val Asn Val Thr Asn Asp
 660 665 670

Val Leu Thr Asn Lys Lys Lys Ile Glu Thr Lys Tyr Asp Glu Lys Glu
 675 680 685

Glu Val Glu Glu Glu Gly Asn Asn Lys Asp Ile His Arg Leu Leu Ser
 690 695 700

Arg Val Ala Leu Asn Ser Phe Leu Thr Leu Asn Ile Leu Gln Asp Thr
 705 710 715 720

Arg Ile Arg Leu Phe Lys Thr Asp Tyr Ile Ala Cys Pro Ser Cys Gly
 725 730 735

Arg Thr Leu Phe Asn Ile Gln Glu Thr Thr Lys Lys Ile Met Lys Leu
 740 745 750

Thr Gly His Leu Lys Gly Val Lys Ile Ala Val Met Gly Cys Ile Val
 755 760 765

Asn Gly Ile Gly Glu Met Ala Asp Ala His Phe Gly Tyr Val Gly Ser
 770 775 780

Ala Pro Lys Lys Ile Asp Leu Tyr Tyr Gly Lys Glu Leu Val Glu Arg
 785 790 795 800

Asn Ile Pro Glu Glu Glu Ala Cys Asp Lys Leu Ile Glu Leu Ile Lys
 805 810 815

Lys His Asn Lys Trp Lys Asp Pro
 820